

CUTICULAR PIGMENT CHANGES IN WORKER
YELLOWJACKETS (HYMENOPTERA: VESPIDAE)KENNETH G. ROSS¹

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Abstract.—Unusual cuticular marks on the yellow regions of the gastral terga of worker yellowjackets of two species (*Vespula vulgaris* and *V. maculifrons*) were studied. These resembled marks found on the gastral terga of physogastric *Vespula* foundresses. The marks were associated with ovarian development among workers of queenless *V. vulgaris* colonies, and the change in structure of pigment granules was similar to that occurring in physogastric queens. Cuticular marks of *V. maculifrons* workers from queenright colonies were not related to ovarian development and the constituent pigment of the marks differed from that of foundresses and laying workers. The evidence presented supports the hypothesis of Ishay and Shimony that changes in structure of the pigment granules of ovipositing wasps are due to components of the granules being shunted to the production of nucleic acids.

Many social wasps of the subfamily Vespinae are aposematically colored with bright yellow or orange pigment on a contrasting dark background. The development of characteristic dark blotches on the otherwise lightly pigmented regions of the cuticle of physogastric queens is well known among these wasps (Heldmann, 1934; Spradbery, 1973). These marks typically appear medially on the posterior portions of gastral terga I-IV in late season foundresses (Edwards, 1980); their cause has variously been attributed to external wear, precipitation of pigments due to friction between sclerites, or oxidation of the pigments due to diffusion of substances through the cuticle (Marchal, 1896; Becker, 1937a; Spradbery, 1973). Ishay and Shimony (1982) presented evidence that the cuticular marks result from loss and change in structure of the pigment granules (xanthosomes) comprising the marks. The pigment granules are thought to contain pteridines (Becker, 1937b) which may be shunted to the production of nitrogenous bases for DNA synthesis.

In this paper I report the occurrence of anomalous dark marks on the yellow regions of the gastral tergites of workers of *Vespula vulgaris* (L.) and *V. maculifrons* (Buysson) similar to the marks found on aged queens. Evidence is presented to suggest that the mechanisms giving rise to the marks in workers are of two distinct types, with one apparently analogous to that occurring in aged queens.

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Table 1. Colony composition and population of five *Vespula* study colonies.

Colony	Date collected	Population			Total	Foundress present	Size of worker sample taken
		♀	♂	♀			
<i>V. vulgaris</i>							
V10	Aug. 27	1,420	—	—	1,420	no	80
V16	Sept. 16	713	96	—	809	no	79
V35	Nov. 1	15	—	—	15	no	15
<i>V. maculifrons</i>							
M26	Oct. 8	1,164	454	165	1,783	yes	80
M29	Oct. 15	1,215	552	154	1,921	yes	60

MATERIALS AND METHODS

Three colonies of *Vespula vulgaris* and two of *V. maculifrons* were collected during late summer and fall of 1982 in Tompkins County, New York. These colonies were part of a larger *Vespula* colony survey in which 35 colonies of five species were sampled; they were chosen for further study on the basis of observed cuticular anomalies of workers. The colonies were lightly anesthetized with ethyl ether, excavated, and transported to the laboratory in large plastic bags. The colonies were killed by freezing. Adults were counted and sorted according to sex and caste; colony composition and population are presented in Table 1. Workers and queens were held in a freezer at -10°C until subsequent examination.

Among the study colonies, the *V. vulgaris* colonies were queenless at the time of collection, while the *V. maculifrons* colonies possessed functional queens (Table 1). Queenlessness was inferred from non-recovery of a foundress, presence of workers with well developed ovaries, and presence of supernumerary eggs in the cells (Ross and Visscher, 1983).

The inner and outer surfaces of gastral terga of workers and queens were studied using light and scanning electron microscopy. The inner fold of the cuticle of the sclerites was teased away and the specimens were coated with a thin (250–350 Å) layer of gold-palladium in a Balzers® sputter coater. The external and internal surfaces of the terga were observed with a AMR® Model 1000 SEM at 10 kilovolts.

Random samples of non-teneral workers from within the colonies were selected for wing wear analysis and dissection of the ovaries. Wing wear was determined subjectively on a zero (no wear) to three (greatest wear) scale. Degree of development of gastral cuticular marks was similarly rated on a zero (no marks) to two (extensive marks) scale. Ovarian development was assessed using a modification of Cumber's (1949) ovariole index.

Data were descriptively analyzed with Exploratory Data Analysis (Velle-

Table 2. Frequency of occurrence of anomalous cuticular marks on the gasters of workers of five *Vespula* colonies. Percentages of total are in parentheses.

Colony	No marks	Number of workers with	
		Intermediate development of marks	Strong development of marks
V10	72 (90%)	6 (7.5%)	2 (2.5%)
V16	52 (65.8%)	1 (1.3%)	26 (32.9%)
V35	11 (73.3%)	—	4 (26.7%)
M26	72 (90%)	—	8 (10%)
M29	43 (71.7%)	7 (11.7%)	10 (16.7%)

man and Hoaglin, 1981). When significant trends were suggested, data were further analyzed using conventional statistical tests.

RESULTS

Workers in the five study colonies possessed dark cuticular marks on the yellow portion of one or more gastral terga (Fig. 1). The frequency and degree of development of the marks among colony workers are shown in Table 2. The marks appeared identical to those of physogastric queens when the isolated terga were viewed under a dissection microscope from internal and external aspects. The marks occurred on the posterior portions of the terga and were most prominent medially; they consisted of areas of reddish-brown pigment bordered by dark bands. Immediately adjacent were areas of apparently normal yellow pigment. Clear spots throughout the marks represent the columnae (cuticular pillars between the infolding cuticle, see Becker, 1937b; Shimony and Ishay, 1981). The layer of reddish-brown pigment comprising the cuticular mark was dry, thin, and brittle and was easily removed as flakes, while adjacent layers of normal yellow pigment were considerably thicker and more malleable. Concentric dark rings throughout the reddish-brown area, conspicuous in physogastric queens (Fig. 1a), were absent from the marks of workers. Cuticular marks were not found in males or fall queens from the study colonies, nor were they found in workers randomly sampled from 30 other colonies of *V. vulgaris*, *V. maculifrons*, *V. germanica* (F.), *V. flavopilosa* Jacobson, or *V. vidua* (Saussure) collected during the same season.

For *V. maculifrons* workers the marks were most commonly found on gastral tergum I (Fig. 1c), but occasionally also on T II. Many of these workers possessed dark discolorations of the lateral and ventral yellow regions of the gaster as well. For *V. vulgaris* workers the marks occurred on gastral terga I–V and were often spread laterally along the posterior margin of the segment

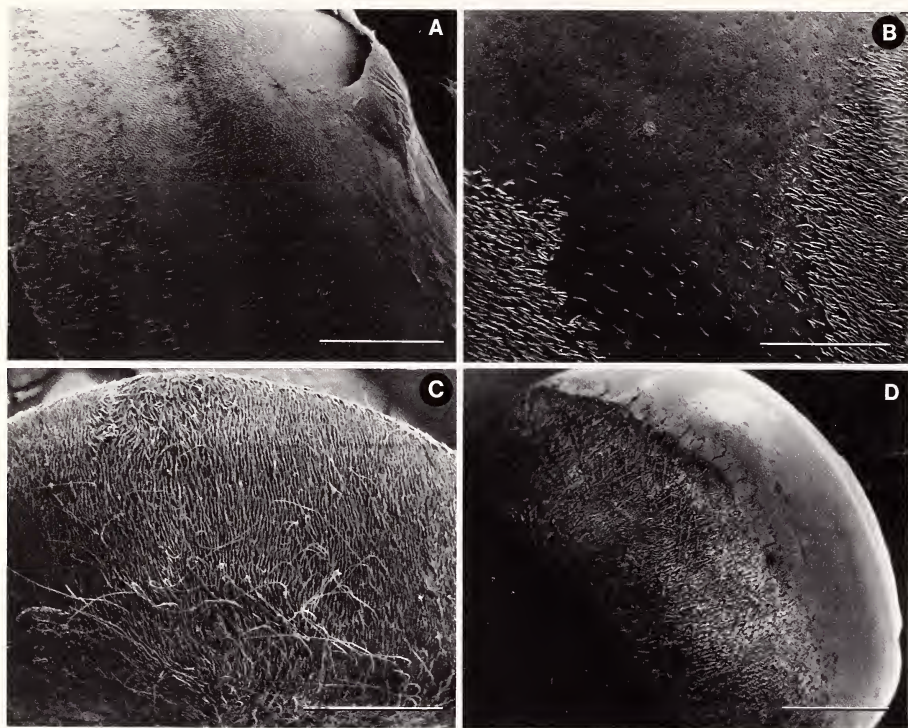


Figs. 1a-c. 1a. Cuticular marks on gastral tergites of physogastric queen of *V. maculifrons* (arrows). The yellow regions are faded in contrast to fall or spring queens ($\times 6.6$, bar = 4.0 mm). 1b. Cuticular marks on gastral tergites of laying worker of *V. vulgaris* (arrow). The yellow regions of the body are dull and faded in contrast to nestmates without developed ovaries ($\times 8.2$, bar = 3.0 mm). 1c. Anomalous cuticular mark on gastral tergite I of *V. maculifrons* worker (arrow) ($\times 8.2$, bar = 3.0 mm).

(Fig. 1b). These workers also commonly exhibited extensive lateral discoloration. The yellow pigment of *V. vulgaris* workers with marks was quite dull and faded in comparison to other nestmates, as is common for physogastric foundresses. In contrast, the yellow pigment of *V. maculifrons* workers with gastral cuticular marks was characteristically bright.

Many workers in the three queenless *V. vulgaris* colonies had developed ovaries (Ross, unpublished data) and were acting as functional reproductives. The presence and degree of development of cuticular marks were positively associated with ovarian index for workers in these colonies (ANOVA, $F = 40.7, 23.2, \text{ and } 103.6$; all $P < 0.005$). Ovarian development was uncommon among workers in the two queenright *V. maculifrons* colonies and was not associated with development of gastral cuticular marks. Presence of the marks was not associated with wing wear in workers of the five study colonies.

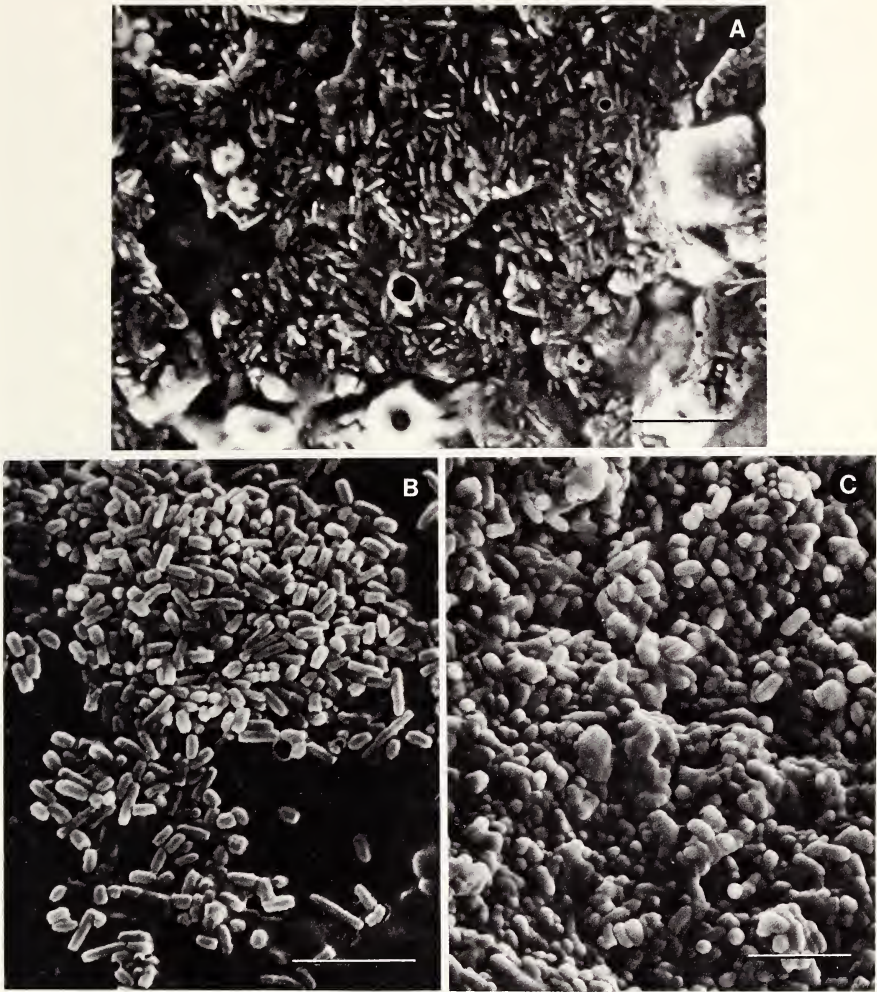
Scanning electron micrographs revealed loss of setae and hairs from the gastral terga of laying workers of *V. vulgaris*, similar to the loss of these structures in physogastric queens (Figs. 2a, b). Loss of the hair and setae occurred on the same segments as did cuticular marks. However, the areas of hair loss did not correspond to the exact areas of the cuticular marks.



Figs. 2a-d. 2a. Hair and seta loss on gastral tergum of laying worker of *V. vulgaris* ($\times 69$, bar = 0.5 mm). 2b. Hair and seta loss on portion of gastral tergum of physogastric *V. maculifrons* queen. Photograph shows the region in which the cuticular mark is found ($\times 74$, bar = 0.5 mm). 2c. Gastral tergum I of *V. maculifrons* worker with cuticular mark. Posterior of segment is to the top ($\times 78$, bar = 0.5 mm). 2d. Gastral tergum of virgin *V. maculifrons* queen collected in autumn. Posterior of segment is to the left ($\times 30$, bar = 1.0 mm).

Indeed, hair loss of physogastric queens often involved the entire yellow region of any given tergum. Workers of *V. maculifrons* with cuticular marks and virgin queens collected in autumn exhibited an abundance of hair and setae on the gastral tergites (Figs. 2c, d).

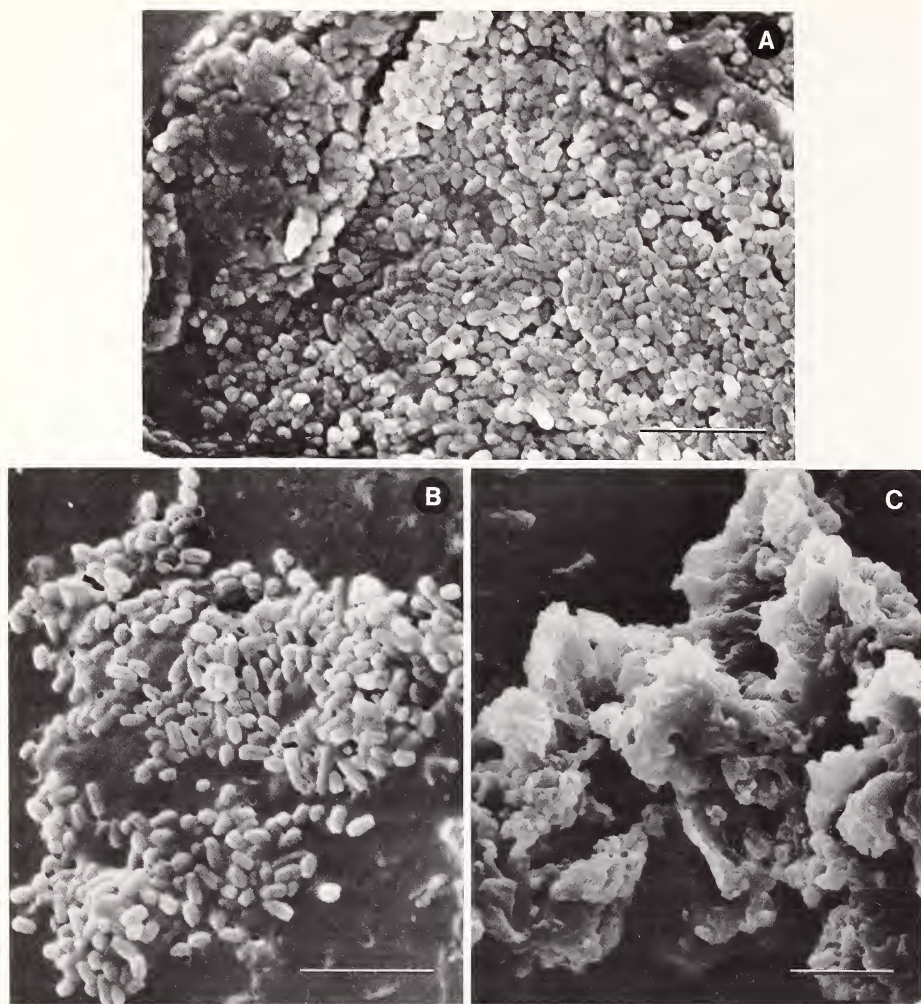
In virgin *V. maculifrons* queens, pigment granules were deposited in dense layers throughout the yellow portion of the tergum. The granules were cylindrical and elongate (ca. $0.8\ \mu\text{m}$ long, Fig. 3a), as shown by Shimony and Ishay (1981) and Ishay and Shimony (1982) for various vespine species. Granules from the yellow pigmented areas surrounding the marks of physogastric queens were more sparsely distributed; many of the granules were shorter and more barrel-shaped than typical granules from virgin queens (ca. $0.6\ \mu\text{m}$ long, Fig. 3b). All granules from the areas of the cuticular marks



Figs. 3a-c. 3a. Pigment granules from yellow region of gastral tergum of virgin *V. maculifrons* queen ($\times 8,300$, bar = $3\ \mu\text{m}$). 3b. Pigment granules from yellow region of gastral tergum of physogastric *V. maculifrons* queen ($\times 10,300$, bar = $3\ \mu\text{m}$). 3c. Pigment granules from area of gastral cuticular mark of physogastric *V. maculifrons* queen ($\times 8,600$, bar = $3\ \mu\text{m}$).

of physogastric queens lost their cylindrical structure to an even greater extent and became barrel-shaped or spherical (Fig. 3c).

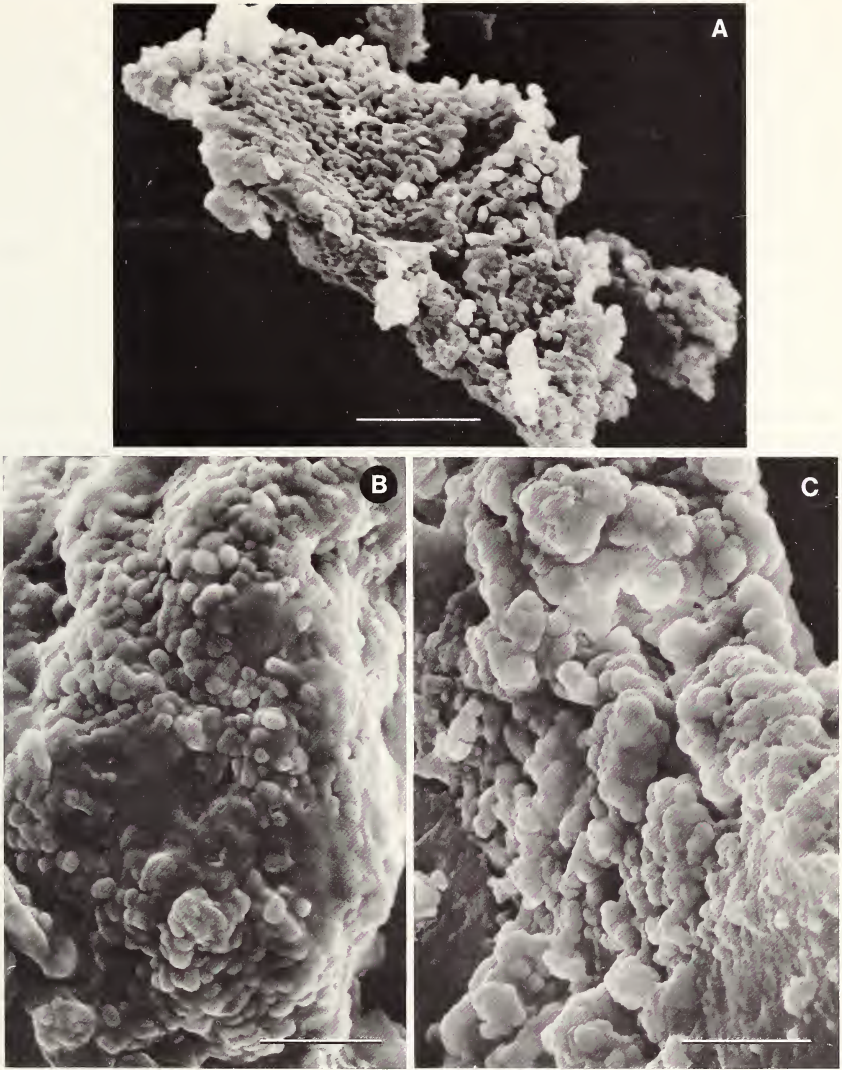
Yellow pigment granules from normal *V. maculifrons* workers exhibited a short, cylindrical structure (ca. $0.6\ \mu\text{m}$ long, Fig. 4a). Granules from the yellow-pigmented regions adjacent to cuticular marks of *V. maculifrons*



Figs. 4a-c. 4a. Pigment granules from yellow region of gastral tergum of normal *V. maculifrons* worker ($\times 10,500$, bar = $3\text{ }\mu\text{m}$). 4b. Pigment granules from yellow region of tergum of *V. maculifrons* worker with gastral cuticular mark ($\times 10,900$, bar = $3\text{ }\mu\text{m}$). 4c. Pigment mass from area of gastral cuticular mark of *V. maculifrons* worker ($\times 8,500$, bar = $3\text{ }\mu\text{m}$).

workers resembled granules from normal conspecifics in size and shape (Fig. 4b). Granules from the area of the cuticular mark lost their discrete, cylindrical character and formed amorphous masses of pigment (Fig. 4c).

Pigment granules from the yellow regions of gastral terga of normal *V. vulgaris* workers were cylindrical (ca. $0.6\text{ }\mu\text{m}$ long, Fig. 5a) and occurred in dense layers. As in physogastric queens, pigment granules from the yellow



Figs. 5a-c. 5a. Pigment granules from yellow region of gastral tergum of normal *V. vulgaris* worker ($\times 10,300$, bar = $3\ \mu\text{m}$). 5b. Pigment granules from yellow region of gastral tergum of laying *V. vulgaris* worker ($\times 10,150$, bar = $3\ \mu\text{m}$). 5c. Pigment granules from area of cuticular mark on gastral tergum of laying *V. vulgaris* worker ($\times 10,600$, bar = $3\ \mu\text{m}$).

portions of terga of laying workers appeared less dense in their distribution, and the granules became barrel-shaped or spherical (Fig. 5b). Granules from the areas of the cuticular marks lost their discrete structure to some extent and were without exception spherical or irregularly shaped (Fig. 5c).

DISCUSSION

How do the results of this study bear on hypotheses of the origin of cuticular marks on the gastral terga of foundress queens? As suggested by Ishay and Shimony (1982), development of cuticular marks in queens is associated with changes in the structure of the pigment granules found in the yellow regions of the wasps, and this change in the granules is associated with ovarian development. *V. vulgaris* workers with marks from the present study resembled physogastric queens in this altered structure of the yellow pigment granules, particularly in the area of the dark marks. These workers also exhibited significant development of the ovaries and loss of gastral hair and setae (probably resulting from friction between the gaster and cell wall during oviposition). These data suggest that the mechanisms underlying the development of cuticular marks in queens and in laying workers are identical. The development of cuticular marks in laying workers was first reported by Marchal (1896) for *Dolichovespula media* (Retzius) and is probably common to all laying vespine workers.

Spradbery (1973) suggested that cuticular marks in queens result from diffusion of substances through the normally impermeable cuticle via the sockets of worn-away hairs and setae. This hypothesis cannot be ruled out by the results of the present study, although two observations diminish the likelihood of its validity. First, the hair and seta loss in laying workers was not as dramatic as in physogastric queens, yet cuticular marks in workers were equally well developed. Secondly, the outline of areas of hair loss did not approximate the outline of the cuticular marks.

Becker (1937a) suggested that the cuticular marks of queens develop in response to friction between adjacent margins of gastral tergites due to distension of the physogastric queen's gaster. I observed no such friction or contact between tergal margins of laying workers, so this hypothesis also seems unlikely.

The development of cuticular marks in vespine queens is postulated by Ishay and Shimony (1982) to result from the conversion of pteridines in the yellow pigment granules to purines required for the synthesis of DNA. The conversion of the pteridine components of the granules results in the observed change in the structure of the granules. Significant amounts of nucleic acids presumably need to be manufactured for the large number of eggs produced by late-season foundresses. My findings that structural changes in the granules accompany ovarian development and oviposition in workers, as well as in queen yellowjackets, support this hypothesis.

The development of cuticular marks in *V. maculifrons* workers from the present study seems unrelated to the development of these marks in ovipositing wasps. The *V. maculifrons* workers with marks exhibited no significant ovarian development or loss of hair and setae from the gastral terga, and these workers did not differ from normal nestmates in level of foraging

activity (deduced from amount of wing wear). Cuticular marks developed primarily on gastral tergum I in *V. maculifrons* workers, while these were most prominent on the more posterior segments of laying workers of *V. vulgaris* and of physogastric queens. The change in structure of the pigment granules from the marks of *V. maculifrons* workers seems to differ fundamentally from that occurring in ovary-developed queens and workers; granules from *V. maculifrons* exhibited virtually none of the discrete structure normally visible. Finally, the marks were confined to a significant proportion of the workers from only two *V. maculifrons* colonies collected; these colonies did not differ in any obvious respects from 13 other conspecific colonies collected during the same season. From these data I conclude that the cuticular marks of the *V. maculifrons* workers resulted from an anomaly during deposition of the yellow pigment layer, perhaps due to a genetic mutation. Ishay and Shimony (1982) report similar aberrations in patterns of pigmentation and structure of the granules in *Vespa orientalis* F. workers and males.

If components of the yellow pigment granules are indeed shunted to production of DNA in ovipositing wasps, then the pigment may be regarded as a vital storage substance. Given the variable proportions of yellow coloration in relation to dark coloration among vespine species, this storage product may be more limiting in some species than in others. Indeed, we may predict that the development of cuticular marks in those species with relatively low proportions of yellow-pigmented cuticle (e.g. *V. vulgaris*, *V. flavopilosa*) will be more extensive than in those species with high proportions of yellow-pigmented cuticle (e.g. *V. germanica*). My preliminary observations suggest that this prediction will hold true.

ACKNOWLEDGMENTS

I thank Mary Ann Moran and James M. Carpenter for reviewing the manuscript. Logistic and financial support was provided by Roger A. Morse of Cornell University's Dyce Honey Bee Laboratory and by the Departments of Entomology at Cornell University and the University of Georgia. Valuable technical assistance with SEM work was provided by Mary Kay Campenot.

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Received April 29, 1983; accepted August 17, 1983.